



Rainbow Trout Milt Cryopreservation Protocol

April, 2004

Rainbow trout milt is obtained by stripping the males and collecting the sample in a ziploc sandwich bag. The bag is filled with oxygen gas and placed in a 5°C cooler for transport to the laboratory.

Upon receipt, the sperm motility is assessed visually using phase contrast microscopy (400 x) and rated with a motility score of 0 to 5, with 0 being no motile cells. To assess motility, a drop of water (12°C) is placed on a microscope slide and secured on the microscope stage. A glass Pasteur pipette is then dipped into the milt and quickly mixed into the water. The sperm motility is assessed immediately, without a coverslip.

Samples are then diluted 1:3 (v:v) with cryopreservation media at 12°C. The samples are loaded into 0.5ml straws and frozen using the Cryo Bio System Mini Digitcool UJ400 (IMV Corporation, Minneapolis, MN) with the following curve: 5°C to -70°C at -30°C per minute and then plunged in liquid nitrogen for storage.

Samples are thawed for 1 minute in a 12°C water bath and motility analysis is performed as described previously.

Recipe:

Cryopreservation media

300mM Glucose
10% DMSO (by volume)
13.30% Egg Yolk (by volume)
pH of 8.0 to 8.5.

Reference:

Coson, J. et al. 1999. In: The Male Gamete. Ed: C. Gagnon